UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/532,258	10/02/2006	J. Keith Joung	62031(51588)	8615

71284 7590 01/19/2011

EDWARDS ANGELL PALMER & DODGE LLP
P.O. BOX 55874
BOSTON, MA 02205

EXAMINER	
LIU, SUE XU	

ART UNIT	PAPER NUMBER
1639	

MAIL DATE	DELIVERY MODE
01/19/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/532,258	JOUNG ET AL.	
	Examiner	Art Unit	
	SUE LIU	1639	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *7/20/10*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7,9,10,12-35,37-39 and 96-98* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7,9,10,12-35,37-39 and 96-98* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *7/20/2010; 8/5/2010* .
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/20/2010 has been entered.

Claim Status

2. Claims 8, 11, 36 and 40-95 have been cancelled.

3. Claims 96-98 have been added as filed on 7/20/2010.

Claims 1-7, 9, 10, 12-35, 37-39 and 96-98 are currently pending.

Claims 1-7, 9, 10, 12-35, 37-39 and 96-98 are being examined in this application.

Election/Restrictions

4. Applicant's election with traverse of Group 1 (claims 1-39) in the reply filed on 8/28/08 is previously acknowledged. The newly added claims 96-98 are grouped with the previously elected Group 1 invention.

5. Applicant's election of the following species:

A.) three zinc finger;

B.) Cys2His2;

in the reply filed on 8/28/08 is as previously acknowledged.

Priority

6. This application is filed under 35 U.S.C 371 of PCT/US03/34010 (filed on 10/23/2003), which claims priority to US provisional applications 60/420,458 (filed on 10/23/2002) and 60/466,889 (filed on 04/30/2003).

Information Disclosure Statement

7. The IDS filed on 7/20/2010 and 8/5/2010 have been considered. See the attached PTO 1449 forms.

Specification

8. The specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification. MPEP 608.01.

Claim Objection(s) / Rejection(s) Withdrawn

9. All previous claim Objection(s) / Rejection(s) as set forth in the previous Office action (mailed 1/20/2010) that are not repeated and/or maintained in the instant Office action are withdrawn.

New/Maintained Claim Objection(s) / Rejection(s)

Claim Rejections - 35 USC § 112

10. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Second paragraph of 35 U.S.C. 112

11. Claims 1-7, 9, 10, 12-35, 37-39 and 96-98 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 recites the limitation "the target site construct" in line 4 of step a). There is insufficient antecedent basis for this limitation in the claim. In line 1 of step a), the instant claim 1 recites "target site constructs" in the plural. It is not clear to which of the plurality of "target constructs" the said term in line 4 of step a) is referring.

Claim 1 also recites "for binding to subsites that together comprise the sequence of interest" in the last line of Step c), which is unclear. It is not clear to which "subsites" the said term is referring.

Claim 97 recites "the high-stringency conditions comprise one or more of a lower salt concentration…" which phrase is unclear and confusing. It is not clear if the said claim is reciting the "high-stringency conditions" comprise additional "salts" comparing to the "low-stringency conditions", or if the "high stringency conditions" have "lower" or "higher" salt concentrations than the "low stringency conditions."

Scope of Enablement Rejection

12. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

13. Claims 1-7, 9, 10, 12-35, 37-39 and 96-98 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for using "low stringent condition" and "high stringent conditions" that are have routine incubation temperature for the zinc finger selection steps, does not reasonably provide enablement for using "high-stringent conditions" that encompass incubating zinc finger proteins at high temperature such as 65°C or above (as explicitly recited in Claim 97 and Spec., [0073]). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. §112, first paragraph, have been described In re Wands, 8 USPQ2d 1400(1988). They are:

1. The breadth of the claims;
2. The nature of the invention;
3. The state of the prior art;
4. The predictability or lack thereof in the art
5. The level of skill in the art;
6. The amount of direction or guidance present;
7. The presence or absence of working examples;
8. The quantity of experimentation needed.

The nature of the invention / The breadth of the claims

The nature of the invention in claim 1 and dependent claims is a method of generating screening for zinc-figure proteins that can bind to a target sequence of interest using various method steps/reagents. The nature of the invention in claim 97 is a method of screening for desired zinc-figure protein using high stringent conditions having a certain temperature range, salt concentration, etc. The breadth of the instant claims (especially the broad claim 1) encompasses any method steps and/reagents. No structural and/or functional limitations are provided for the claimed genuses of method steps/reagents especially the various "low" and "high" stringent conditions. The instant specification broadly defines the said conditions (e.g. [0073]), which definitions can encompass infinite number of different conditions. As recited in claim 97 and paragraph [0073] of the specification, the "high stringent condition" can "typically comprise lower salt concentrations, a temperature of 65°C."

The state of the prior art/ The predictability or lack thereof in the art

The state of the art does not provide that it is predictable to screen zinc finger proteins using protein-DNA binding assays under any "condition." The condition under which protein can be active in its native state is limited. It is especially true for incubation temperatures under which a given protein can exist in its active (i.e. binding) state or it denaturing (i.e. non-binding) state. In fact, the state of art teaches that the majority of proteins would be inactivated if heat treated such as heating to above 65°C. For example, **Rousselle** et al., (Journal of Biological Chemistry. Vol.270 (23): 13766-13770; 1995) teach that proteins are heat denatured at above

65°C (e.g. Abstract). That is the zinc finger proteins would be denatured when heated to 65°C (similar to the majority of other cellular proteins), and would not be able to bind to their target DNA sequences. Thus, the instant claimed methods cannot be performed. The instant specification does not provide any example of using the "high stringent condition" with the 65°C incubation temperature for binding zinc finger protein to its target DNA sequence. Thus, it is highly unpredictable for one of skilled in the art to perform the instant claimed method of screening.

The level of one of ordinary skill

The level of skill would be high, most likely at the Master or Ph.D. level.

The amount of direction or guidance present / The presence or absence of working examples

The instant specification does not provide any example of using the "high stringent condition" with the 65°C incubation temperature for binding zinc finger protein to its target DNA sequence. The instant specification only briefly and generally state that the "high-stringency conditions typically comprise lower salt concentrations, a temperature of 65°C or greater…" (spec., [0073]), and does not provide any other specific guidance on how the claimed assay can be performed under the said denaturing condition.

The quantity of experimentation needed

Due to the unpredictabilities of assaying proteins (including zinc finger proteins) under various conditions (such as protein denaturing conditions), undue experimentation would be

required. The art has not demonstrated that any "low" or "high" stringent condition can be used to successfully assay protein-DNA binding. In a more narrowing scope, the art provides evidence that incubating proteins under conditions having temperature 65°C or above would denature (or destroy) the protein that that its proper function cannot be performed.

Conclusion

Therefore based on the evidences as a whole regarding each of the above factors (e.g. factors 1-8), the specification, at the time the application was filed, does not satisfy the enablement requirement for the instant claimed method.

Claim Rejections - 35 USC § 103

14. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

15. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Isalan and Others I

16. Claims **1-7, 9, 10, 12-14, 16-19, 21, 22, 26-29, 32-35**, **37** and **96** are rejected under **35 U.S.C. 103(a)** as being unpatentable over **Isalan** et al. (Nature Biotechnology. Vol.19: 656-660;

7/2001; cited previously), and in view of **Choo** et al. (WO 00/27878; 5/18/2000; cited previously), **Wang** et al. (PNAS. Vol.96: 9568-9573; 8/1999), and/or in view of Choo et al. (WO99/47656; 9/23/1999; Referred to as **Choo II**).

The instant claims recite "A method of selecting a multi-zinc finger polypeptide that binds to a sequence of interest comprising at least two subsites, said method comprising the steps of:

a) incubating position-sensitive primary libraries with target site constructs under low-stringency conditions sufficient to form first binding complexes, wherein said primary libraries comprise multi-zinc-finger polypeptides having one variable finger and at least one anchor finger, and wherein the target site construct has one subsite with a sequence identical to a subsite of the sequence of interest, and one or more subsites with sequences to which the anchor finger(s) bind with low affinity;

b) isolating pools comprising nucleic acid sequences encoding the multi-zinc-finger polypeptides having one variable finger, that formed in the first binding complexes of step a) with the target site constructs;

c) recombining the nucleic acid sequences encoding the one variable finger from the isolated pools of step b) to produce a secondary library encoding multi-zinc-finger polypeptides having zinc-fingers partially optimized for binding to subsites of the sequence of interest;

d) incubating the secondary library of step c) with the sequence of interest under high-stringency conditions sufficient to form second high-affinity binding complexes between the multi-zinc-finger polypeptides and the sequence of interest; and

e) isolating nucleic acid sequences encoding multi-zinc-finger polypeptides that formed in the second binding complexes of step d)."

Isalan et al, throughout the publication, teach methods of generating recombinant zinc fingers based on selecting mutant zinc finger proteins that bind to a predetermined nucleic acid sequence (e.g. Abstract; pp.656+).

For **claim 1** step a): *"incubating position*-sensitive primary libraries with target site constructs under low-stringency conditions sufficient to form first binding complexes, wherein said primary libraries comprise multi-zinc-finger polypeptides having one variable finger and at least one anchor finger, and wherein the target site construct has one subsite with a sequence identical to a subsite of the sequence of interest, and one or more subsites with sequences to which the anchor finger(s) bind": This method step is interpreted to mean binding libraries of zinc finger containing proteins to nucleic acid target constructs containing "an anchor site" and a site with a nucleic acid "sequence of interest" (or a predetermined nucleic acid sequence). The reference teaches generating libraries (at least two libraries) of randomized zinc finger proteins, where a wildtype finger is combined with two randomly mutated zinc fingers (e.g. Figure 1; pp.656-657), which the wildtype finger reads the "anchor finger" and the mutated fingers read on the "variable finger". The reference also teaches binding the said libraries of zinc finger proteins to a construct comprising a "predetermined DNA sequence" (e.g. p.657; Figure 2; Table 1 shows an example of using a HIV promoter sequence as the predetermined DNA sequence) and a segment of DNA sequence that binds to the wild-type zinc finger, which the predetermined DNA sequence reads on "a sequence of interest", and the wild-type zinc finger binding sequence reads on the "anchor finger" binding sequence. The instant specification broadly defines the term" position-sensitive" (e.g. Spec. p.6, lines 5+), which can be reasonably interpreted to be any zinc finger libraries that have multiple zinc finger domains (that may have interactions). The reference teaches the generated zinc fingers of the proteins interact with other to achieve "comprehensive DNA recognition" (e.g. p.657), which reads on the inherent property of "position-sensitive".

The instant specification broadly defines the term "low stringency" to "conditions" "which are conducive to the formation of 'binding complexes' comprising both weakly- and strongly-bound proteins and nucleic acids" (spec. PGPUB, [0073]), which definition broadly encompass any condition depending on the point of reference. Similarly, the term "high-stringency conditions" is defined as conditions "which are conducive to the formation of 'high affinity binding complexes' comprising only strongly-bound proteins and nucleic acids." (spec. PGPUB, [0073]), which is also a broad and relative definition. For example, a condition can be considered to be both "low" and "high" stringency depending on the point of reference. That is comparing to another lower stringency condition, a condition maybe considered "high", and comparing to a higher stringency condition, the same condition maybe considered "low". Thus, the selection conditions taught in the Isalan reference can be considered as either low or high stringency condition.

Similarly, the instant specification also broadly defines the phrases "low" and "high" affinity" in relative terms. The instant specification recites "Two molecules that bind strongly to each other have a 'high affinity' for each other, while molecules that bind weakly to each other have a 'low affinity' for each other." (Spec., PGPUB, [0063]). These said definitions given the broadest and reasonable interpretation can encompass any "affinity", because any given affinity can be considered as either "low" or "high" depending on the point of reference. The reference teaches the "anchor finger" and the "variable finger" have affinity to their binding sites (as exhibit through binding interaction; e.g. p.658, left col.), and thus the reference's teachings read on the "low affinity" or "high affinity" binding. In addition, the reference teaches the anchor fingers to be of the same structure as the instant claimed anchor finger (which is also derived

from Zif268), and thus the reference's teachings read on the inherent properties of low affinity and/or specificity.

For **claim 1** step (b): *"isolating pools* comprising nucleic acid sequences encoding the multi-zinc-finger polypeptides having one variable finger, that formed in the first binding complexes with the target site constructs in step a)": The instant claim 1 step (b) is interpreted to mean isolating nucleic acids (molecules) that encode for the zinc finger proteins that bind to the "target site constructs". The reference teaches isolating or selecting the polypeptides that bind to the target constructs through, for example, phage display selection (e.g. Figure 1; p.657), which the selected phage would comprise the DNA encoding for the selected zinc finger polypeptides.

For **claim 1** step (c): *"recombining the nucleic acid sequences encoding* the variable fingers from the isolated pools of step b) to prod*uce a secondary library..."*: The reference teaches recombining the DNA sequences (encoding for the zinc finger polypeptides) of the selected two libraries of zinc fingers (e.g. Figure 1; p.657, left col.), which the resulting library (read on the secondary library) would inherently be "partially optimized for binding…"

For **claim 1** step (d): "incubating the secondary library of step c) with the sequence of interest under high-stringency conditions sufficient to form second high affinity binding complexes*...":* The reference also teaches binding the recombined zinc finger polypeptides with the predetermined sequence through additional rounds of selection (e.g. Figure 1; Table 1; pp.657-658), which selection would inherently result in high affinity binding proteins (see more discussion above).

For **claim 1** step (e): *"isolating nucleic acid sequences encoding multi-zinc finger polypeptides that formed in the second binding complexes... ":* The reference teaches isolating nucleic acids (molecules) that encode for zinc finger proteins that bind to the predetermined sequence of interest (e.g. pp.657+; Figure 1).

For **claims 2** and **3**: The reference teaches at least two or three zinc fingers (e.g. Figures 1 and 2).

For **claim 4**: The reference teaches the target construct having the predefined sequence (or DNA sequence of interest) (e.g. Figure 2; Table 1).

For **claim 5**: The reference teaches various numbers base pairs (such as 3 bps) at the zinc finger binding DNA sequence (e.g. Figure 2 and Table 1).

For **claims 6** and **7**: The reference teaches various numbers (such as 3 sites for three fingers) of binding sites (e.g. Figure 2 and table 1).

For **claim 9**: The reference teaches the wild-type target site (the anchor finger binding sequence) comprises sequence of GCC (e.g. Figure 2A where the binding sequence for Lib23).

For **claims 10, 12-14** and **16-19**: The reference teaches the library of zinc finger proteins comprise wildtype finger sequence from a naturally occurring zinc finger protein, Zif268 (e.g. p.657) as well as phage displayed mutant Zif268 zinc finger proteins (i.e. synthetic derivative) (e.g. Figure 1).

For **claims 21** and **22**: The reference teaches randomizing at least the residues within the α-helical region of the zinc fingers (e.g. Figure 2).

For **claims 27, 28, 34** and **35**: The reference teaches expressing the zinc finger library in bacteriophage system (e.g. p.659).

For **claims 29** and **37**: The reference teaches incubating the phage displayed proteins with the target constructs in test tubes (i.e. in vitro) (e.g. p.660).

For **claim 32**: The reference teaches using PCR to recombine the two libraries of genes encoding for the zinc finger proteins. (e.g. p.660).

For **claim 96**: The reference teaches utilizing phage display, which is not a polysome system.

Isalan et al do not explicitly state using "low stringency conditions" the first rounds of selection, and later using "high stringency conditions" ("wherein the high-stringency conditions are more stringent than the low-stringency conditions") as recited in **claim 1**.

However, **Choo** et al., throughout the publication, teaches various methods of generating mutant zinc finger protein libraries, and methods of screening the libraries (e.g. Abstract). The reference teaches using various screening/selecting conditions including both high and low stringencies so that high or low affinity binding protein can be selected (e.g. pp.27+). The reference also discusses various conditions such as buffer concentrations for different selections (including different ionic strength, detergent concentrations, etc.; pp.25+), which encompass low and/or high stringency conditions. The Choo reference also teaches selection for "low to medium affinity zinc finger polypeptides can be selected" and they "can be superior candidates for generating very high affinity zinc finger polypeptides" in an "affinity sharpening" process through subsequent rounds of selections (e.g. p.27, lines 6+; p.29, lines 15+). The reference also teaches low affinity "target nucleic acids" can be used "to enrich for library members" with "relatively low affinity" (e.g. p.29, lines 9+).

Therefore, it would have been prima facie obvious for one of ordinary skill in the art at the time the invention was made to use various desired selection conditions (low or high stringency) as well as nucleic acid binding constructs to select zinc finger proteins with desired binding affinity.

A person of ordinary skill in the art would have been motivated at the time of the invention to use low stringency conditions with low affinity binding constructs (for the anchor finger) to conduct the initial round of zinc finger protein selection, because Choo et al. teach the advantages of selecting under low stringency condition so that zinc finger with low to medium affinity can be isolated to increase the potential pool of zinc finger protein candidates, as discussed supra. Because the cited references teach methods of selecting/screening zinc finger proteins under various conditions for the purpose of isolating zinc finger with desired binding affinity and specificity, it would have been obvious to one skilled in the art to substitute one selection condition (high stringency) for the other (low stringency) to achieve the predictable result of selecting/screening the desired zinc finger protein.

A person of ordinary skill in the art would have been motivated at the time of the invention to use high stringency conditions to conduct the subsequent rounds of zinc finger protein selection to select for high affinity binding zinc fingers, because Choo et al. teach the advantages of "affinity sharpening" by increasing the selection stringency so that zinc finger with high affinity can be isolated. Thus, it would have been obvious to one of ordinary skill in the art to apply the standard technique of selecting zinc finger proteins using various selection conditions as taught by both Isalan and Choo, to improve zinc finger selection assays for the

predicable result of enabling standard zinc finger protein selection/screening through binding assays. Therefore, it would have been obvious to a person of ordinary skill in the art to try various combinations of the known conditions and/or nucleic acid binding constructs for selecting zinc finger binding proteins, in an attempt to optimize zinc finger protein screening depending on the needs of various routine experimental designs, as a person with ordinary skill has good reason to pursue the known options within his or her technical grasp.

A person of ordinary skill in the art would have reasonable expectation of success of achieving such modifications since the cited references have demonstrated the success of generating various zinc finger protein libraries (or their encoding nucleic acids), using various target binding constructs, using various binding conditions, etc., for selecting the desired zinc finger proteins.

In addition, Isalan et al. do not explicitly teach the libraries of proteins are expressed in vitro as recited in **clms 26** and **33**.

However, **Choo** et al., throughout the publication, teaches various methods of generating mutant zinc finger protein libraries, and methods of screening the libraries (e.g. Abstract). The reference teaches using in vitro polysome display of zinc finger proteins (e.g. p. 22; p.24), which the zinc finger polypeptides are produced in vitro using an in vitro transcription/translation system. The reference also teaches the in vitro protein production method offers various advantages such as improved affinity screening (e.g. p.23, lines 10+).

Wang et al., throughout the publication, teaches selecting for zinc finger proteins that bind to target DNA sequence of interest (e.g. Abstract). The reference teaches using phage display library to select for proteins with specific affinity for specific DNA target (e.g.

pp.9568+). The reference also teaches the selection condition "stringency" is increased at each successive round of selection, and various low/high stringency conditions (including change in temperature, target concentration were used (e.g. p.9568, right col., para 4). The reference also teaches the advantages of selection based on increased stringency (in each successive round of selection) so that proteins the assay can favor "selection of the tightest-binding sequences from each pool" (p.9571, left col., para 1).

Further, **Choo II**, throughout the publication, teach methods of screening/selecting for zinc finger binding protein that have specific affinity for target DNA of interest (e.g. Abstract). The Choo II reference also teaches increasing "selection pressure" (i.e. stringency conditions) at each subsequent round of selection (e.g. p.29, lines 10+; p.33, lines 1+). The reference also teaches the advantages of using multiple rounds of selection with increased stringency so to "enrich the mutant pool for the desired phage and eventually isolate the preferred clone(s)" (p.16, lines 5+) such as identifying proteins that bind to specific DNA with modified nucleotides when using conditions with increased stringency for selection (e.g. p.33).

Therefore, it would have been prima facie obvious for one of ordinary skill in the art at the time the invention was made to generate mutant zinc finger proteins using in vitro expression as well as using selection conditions with increasing stringency.

A person of ordinary skill in the art would have been motivated at the time of the invention to use in vitro expression to produce mutant zinc finger proteins, because Choo et al. teach various advantages of in vitro expression such that a convenient and improved affinity screening methods can be used. In addition, because all of the cited references teach methods of producing mutant zinc finger proteins using various routine and known expression methods, it

would have been obvious to one skilled in the art to substitute one expression method (in vivo production) for the other (in vitro production) to achieve the predictable result of expressing the desired zinc finger proteins.

A person of ordinary skill in the art would have been motivated at the time of the invention to use selection conditions with increasing stringency, because it is routine in the art to increase the selection pressure so that each subsequent round of selection will "narrow" the selection pool and isolate the desired zinc finger protein as taught by all the cited references. As the Wang and Choo II references teach various advantages of using conditions with increasing stringency such as to produce proteins with "tightest" binding, one of ordinary skilled in the art would be motivated to adjust the selection conditions to increase their stringency at each successive selection. In addition, because all of the cited references teach methods of producing mutant zinc finger proteins using various routine and known selection conditions, it would have been obvious to one skilled in the art to substitute one selection condition for the other to achieve the predictable result of selecting for zinc finger proteins with the designed affinity and specificity.

A person of ordinary skill in the art would have reasonable expectation of success of achieving such modifications since all of the cited references have demonstrated the success of generating randomized/mutant zinc finger protein libraries using various protein production techniques.

Isalan and Others II

17. Claims **1-7, 9, 10, 12-14, 16-19, 21-29, 32-35** and **37** are rejected under 35 U.S.C. 103(a) as being unpatentable over **Isalan** et al. (Nature Biotechnology. Vol.19: 656-660; 7/2001), in view of **Choo** et al. (WO 00/27878; 5/18/2000), **Wang** et al. (PNAS. Vol.96: 9568-9573; 8/1999), Choo et al. (WO99/47656; 9/23/1999; Referred to as **Choo II**), and Isalan et al. (Biochemistry. Vol.37: 12026-12033; 1998; referred to as **Isalan II**).

Isalan et al, Choo et al., Wang et al, and Choo II teach methods of generating recombinant zinc fingers based on selecting mutant zinc finger proteins that bind to a predetermined nucleic acid sequence, as discussed supra. The teachings of the Isalan and Choo references as discussed above are hereby incorporated by reference in their entirety.

The combination of Isalan, Choo, Wang and Choo II does not explicitly teach between 16 to 20 amino acids are represented at each of the randomized positions as recited in **clms 23-25**.

However, **Isalan II**, throughout the publication, teach generating various zinc finger proteins using phage display technology (e.g. Abstract). The reference teaches randomizing the desired positions in the zinc finger region (e.g. p.12027). The reference also teaches generating codons for all 20 amino acid residues (e.g. p.12028, left col.). The reference also teaches the need to generate diverse amino acid sequence for the selection process (e.g. 12026).

Therefore, it would have been prima facie obvious for one of ordinary skill in the art at the time the invention was made to generate randomized zinc finger protein libraries comprising various number of possible amino acid residues at each randomized position.

A person of ordinary skill in the art would have been motivated at the time of the invention to represent the desired number of amino acids at each randomized positions in a zinc

finger, because Isalan II teaches the need to generate randomized zinc finger proteins with diverse sequences and it is routine and known to generate libraries that represent various number of amino acids (such as all 20 amino acids). In addition, because both the Isalan references teach methods of generating randomized zinc finger libraries with random amino acid mutation at various positions within the α-recognition region for various screening purposes, it would have been obvious to one skilled in the art to substitute one set of amino acids (that are represented at each position; such as 8 different amino acids) for the another set (such as 16 to 20 amino acids) to achieve the predictable result of generating libraries of randomized zinc fingers representing various amino acids at the mutated positions.

A person of ordinary skill in the art would have reasonable expectation of success of achieving such modifications since all of the cited references have demonstrated the success of generating randomized zinc finger protein libraries with randomized positions representing the desired number amino acids.

Isalan and Others III

18. Claims **1-7, 9, 10, 12-14, 16-19, 21-35** and **37-39** are rejected under 35 U.S.C. 103(a) as being unpatentable over **Isalan** et al. (Nature Biotechnology. Vol.19: 656-660; 7/2001), in view of **Wang** et al. (PNAS. Vol.96: 9568-9573; 8/1999), Choo et al. (WO99/47656; 9/23/1999; Referred to as **Choo II**), Isalan et al. (Biochemistry. Vol.37: 12026-12033; 1998; referred to as **Isalan II**) and **Choo** et al. (WO 00/27878; 5/18/2000), as applied to claims 1-7, 9, 10, 12-14, 16-19, 21-25, 26-29, 32-35 and 37, and further in view of **Joung** et al. (PNAS. Vol.97: 7382-7387; 6/20/2000).

Isalan et al teach methods of generating recombinant zinc fingers based on selecting mutant zinc finger proteins that bind to a predetermined nucleic acid sequence, as discussed supra.

Isalan II, throughout the publication, teach generating various zinc finger proteins using phage display technology, as discussed supra.

Choo et al., throughout the publication, teaches various methods of generating mutant zinc finger protein libraries, and methods of screening the libraries, as discussed supra.

The combined teachings of the Isalan, Isalan II, Choo, Wang et al, and Choo II references as discussed above are hereby incorporated by reference in their entirety.

The combination of the Isalan, Isalan II, Choo, Wang et al, and Choo II references does not explicitly teach the libraries are incubated in cells as recited in **clms 30**, **31**, **38** and **39**.

However, **Joung** et al., throughout the publication, teach methods of generating randomly mutated zinc finger proteins and screening the zinc finger target binding in cells (e.g. Abstract). The reference teaches using a bacterial two hybrid system for zinc finger selection (e.g. Abstract; pp.7383). The reference also teaches the advantages of using such a system so that zinc finger proteins with high affinity can be isolated in a single selection step, and thus allowing a more rapid screening process (e.g. Abstract). The reference also teaches using various selection conditions to select for zinc finger proteins that bind to the target sites (e.g. pp.7383+). The reference also teaches the selection system used is under "stringent standard" and "account for why we isolated such a small number of specific candidates".

Therefore, it would have been prima facie obvious for one of ordinary skill in the art at the time the invention was made to screen for zinc finger polypeptides using an in vivo bacterial system and performing the selection process under high stringency.

A person of ordinary skill in the art would have been motivated at the time of the invention to using a bacterial cell based selection system to screen for target binding zinc finger proteins, because Joung et al. teach the advantages of using a bacteria call based system so that zinc finger proteins with high affinity can be isolated in a single selection step, and thus allowing a more rapid screening process. In addition, because all of the cited references teach methods of screening mutant/randomized zinc finger proteins for binding to nucleic acid targets of interest using various screening techniques, it would have been obvious to one skilled in the art to substitute one screening strategy (in vitro affinity assay) for the other (in vivo bacteria cell based 2 hybrid system) to achieve the predictable result of selecting for desired zinc finger proteins.

A person of ordinary skill in the art would have been motivated at the time of the invention to select zinc finger proteins under high stringency to obtain high affinity binding proteins, because Joung et al. teach the need to perform the selection under high stringency so that zinc finger proteins with high affinity and specificity can be obtained. Thus, it would have been obvious to one of ordinary skill in the art to apply the standard technique of screening/selecting zinc finger proteins under various conditions, to improve and/or optimize the screening/selection assay for the predicable result of enabling standard protein selection that would result in desired proteins with the desired binding affinity/specificity for targets of interest.

A person of ordinary skill in the art would have reasonable expectation of success of achieving such modifications since all of the cited references have demonstrated the success of generating selecting various mutant/randomized zinc fingers using various protein production techniques under various conditions.

Isalan and Others IV

19. Claims **1-7, 9, 10, 12-35** and **37-39** are rejected under 35 U.S.C. 103(a) as being unpatentable over **Isalan** et al. (Nature Biotechnology. Vol.19: 656-660; 7/2001), **Wang** et al. (PNAS. Vol.96: 9568-9573; 8/1999), Choo et al. (WO99/47656; 9/23/1999; Referred to as **Choo II**), Isalan et al. (Biochemistry. Vol.37: 12026-12033; 1998; referred to as **Isalan II**), **Choo** et al. (WO 00/27878; 5/18/2000) and **Joung** et al. (PNAS. Vol.97: 7382-7387; 6/20/2000), as applied to claims 1-7, 9, 10, 11-14, 16-19, 21-35 and 37-39, and further in view of **Chandrasegaran** (US 6,265,196; 7/24/2001).

Isalan et al teach methods of generating recombinant zinc fingers based on selecting mutant zinc finger proteins that bind to a predetermined nucleic acid sequence, as discussed supra.

Isalan II, throughout the publication, teach generating various zinc finger proteins using phage display technology, as discussed supra.

Choo et al., throughout the publication, teaches various methods of generating mutant zinc finger protein libraries, and methods of screening the libraries, as discussed supra.

Joung et al., throughout the publication, teach methods of generating randomly mutated zinc finger proteins and screening the zinc finger target binding in cells, as discussed supra.

The combined teachings of the Isalan, Isalan II, Choo, Joung, Wang et al, and Choo II references as discussed above are hereby incorporated by reference in their entirety.

The combination of the Isalan, Isalan II, Choo, Joung, Wang and Choo II references does not explicitly teach the specific zinc finger sequence as recited in **clms 15** and **20**.

However, **Chandrasegaran**, throughout the patent, teach using various zinc finger protein with various amino acid sequences (e.g. Abstract). The reference specifically teaches using a zinc finger with amino acid sequence "QGGNLVR" for recognizing (or binding) the target sequence of GAA (e.g. col.22, Table 1), which the AA sequence matches SEQ ID NO:3. The reference also teaches designing zinc fingers with the appropriate amino acid sequence for binding to desired DNA target sequence of interest (e.g. col.16).

Therefore, it would have been prima facie obvious for one of ordinary skill in the art at the time the invention was made to generate zinc finger proteins comprising various amino acid sequences including sequences that are known to bind to certain target sites.

A person of ordinary skill in the art would have been motivated at the time of the invention to use generate zinc finger proteins to comprise a desired known sequence such as the sequence in SEQ ID NO:3, because Chandrasegaran teaches the sequence is known to bind to a specific target sequence and it is routine and known to alter the zinc finger sequences for binding to the desired target sites. In addition, because all of the cited references teach generating zinc finger proteins for binding to desired nucleic acid sequence of interest, it would have been obvious to one skilled in the art to substitute one known zinc finger sequence for the other to achieve the predictable result of generating zinc finger polypeptide with the desired AA sequences.

A person of ordinary skill in the art would have reasonable expectation of success of achieving such modifications since all of the cited references have demonstrated the success of generating selecting various mutant/randomized zinc fingers using various protein production techniques under various conditions.

Discussion and Answer to Argument

20. Applicant's arguments have been fully considered but they are not persuasive for the following reasons (in addition to reasons of record). Each point of applicant's traversal is addressed below:

Applicants are respectfully directed to the above new or modified rejections for answer to arguments.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sue Liu whose telephone number is 571-272-5539. The examiner can normally be reached on M-F 9am-3pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joanne Hama can be reached at 571-272-2911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Sue Liu/
Primary Examiner, AU 1639
1/14/2011